Differentiation and growth of kype skeletal tissues in anadromous male Atlantic Salmon (Salmo salar)

P.-ECKHARD WITTEN*1 and BRIAN K. HALL2

1Institute of Marine Research at the University of Kiel, Germany and 2Department of Biology, Dalhousie University, Halifax NS, Canada

ABSTRACT The re-initiation of bone development in adult starving Atlantic salmon (Salmo salar) during their energetically expensive upstream migration is remarkable and deserves closer examination. Dramatic alterations of the skull bones and teeth, most prominently, the development of a kype in males, are widely known but little studied or understood. We describe the microstructure and the cellular processes involved in the formation of the skeletal tissues of the kype. Fresh bone material, obtained from animals migrating upstream was subjected to radiological, histological or histochemical analysis. We show that the kype is, in part, composed of rapidly growing skeletal needles arising at the tip of the dentary. Proximally, the needles anastomose into a spongiosa-like meshwork which retains connective tissue inside bone marrow spaces. Ventrally, the needles blend into Sharpey fiber bone. Skeletal needles and Sharpey fiber bone can be distinguished from the compact bone of the dentary by radiography. Rapid formation of the skeleton of the kype is demonstrated by the presence of numerous osteoblasts, a broad distal osteoid zone, and the appearance of proteoglycans at the growth zone. The mode of bone formation in anadromous males can be described as ‘making bone as fast as possible and with as little material as possible’. Unlike the normal compact bone of the dentary, the new skeletal tissue contains chondrocytes and cartilaginous extracellular matrix. Formation of the skeleton of the kype resembles antler development in deer (a form of regeneration), or hyperostotic bone formation in other teleost fishes, rather than periosteal bone growth. The type of bone formation may be understandable in the light of the animals’ starvation and the energetic costs of upstream migration. However, the structured and regulated mode of bone formation suggests that the skeleton of the kype has functional relevance and is not a by-product of hormonal alterations or change of habitat.

KEY WORDS: Grilse, bone growth, spawning migration, kype, Sharpey fiber bone, chondroid bone

Introduction

Alterations of the bones of the skull and the teeth occur in both sexes of Atlantic salmon (Salmo salar) during migration upstream to spawn; morphological changes were described in detail by Tchernavin (1937, 1938a, 1944) almost two-thirds of a century ago, and more recently by Kacem et al. (1998). Although all bones of the salmon skull change during migration, and although feeding teeth are replaced by a new set of teeth known as breeding teeth, the formation of a kype (hook) on the tip of the lower jaw in male salmon is the most prominent alteration (Fig. 1).1

1  The origins of the term "kype" are obscure. It could derive from "kip" for a hook (1615) of for a point, peak or tip (1815). A "kipper" is anything with a beak. Alternatively, it could come from "kypho" for curved, as in kyphosis for excessive rounding of the shoulders. The term "kyped," meaning hooked, can be traced to Scotland (1848), while a "kipper" is a male salmon in spawning season (Oxford English Dictionary, 2nd ed., 1989), perhaps derived from the fact that "a kip-nosed man, in Scotch, means a man with a turned-up pug nose" (Day, 1887, p. 96).

The kype was the topic of scientific interest long before Tchernavlin drew attention to the changes in the salmon skull. Darwin (1877) was aware that male salmon developed a kype during the breeding season. In his classic treatment, British and Irish Salmonidae, Day (1887) noted that "the cause of the existence of a knob on the lower jaw of male salmon and trout has been a fruitful cause of discussion from the early ages down to the present day" (p. 57).

Despite the fact that the Atlantic salmon is one of the best-studied teleost species, little information about the function of the kype is available. Hutchings and Myers (1987) suggested that male salmon use their kype to compete with other anadromous males.

Abbreviations used in this paper: 1SW salmon, grilse or salmon that have spent one winter at sea before returning to spawn for the first time; BICP/NBT, 5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium; HBQ-staining, Hall-Brunt Quadruple Stain; IgG, Immunoglobulin G; K, condition factor; PBS, Phosphate buffered saline solution; TRAP, Tartrate resistant acid phosphatase; TRIS, 2-Amino-2-hydroxymethyl-1,3-propanediol.
Bone metabolism is basically linked to the animal's phosphorous matrix (Lopez et al., 1999a; Huysseune, 2000; Kacem et al., 1998). Now, fish bone is recognized as a dynamic tissue, displaying resorption and remodeling for growth and for metabolic purposes (Hall, 1975, 1978; Benjamin, 1986, 1988a,b, 1989, 1990; Huysseune and Verraes, 1990; Smith and Hall, 1990; Benjamin and Ralphs, 1991; Hall and Miyake, 2000; Huysseune, 2000). Furthermore, in Osteichthyans, cartilage, bone, and teeth are not regarded as separated entities, clearly defined (Huysseune, 2000). Furthermore, in Osteichthyans, cartilage, bone, and teeth are not regarded as separated entities, clearly defined (Huysseune, 2000). Additionally, in Osteichthyans, cartilage, bone, and teeth are not regarded as separated entities, clearly defined (Huysseune, 2000). Therefore, studies on the mammalian skeleton. Today, similarities and differences between fish bone and mammalian bone are well-defined (Huysseune, 2000). Nonetheless, indications that bone metabolism in salmon migrating upstream is triggered by the needs for calcium (Persson et al., 1998; Witten et al., 2001). The cellular processes involved in fish bone turnover are manifold when compared to similar processes in mammalian bone (Teitelbaum, 2000). They include bone resorption by mononucleated cells, multinucleated osteoclasts, and by osteocytes, and demineralization of bone matrix (Lopez et al., 1976; Hughes et al., 1994a,b; Witten et al., 1999a; Huysseune, 2000; Kacem et al., 2000). Recent studies also demonstrate the metabolic activity of the salmon skeleton, but whether this activation mainly reflects activity in the endoskeleton or in the dermoskeleton (the scales) remains unclear (Persson et al., 1998; Kacem et al., 2000). Despite indications that bone metabolism in salmon migrating upstream is triggered by the needs for calcium (Persson et al., 1999), there is evidence that salmon bone metabolism is basically linked to the animals' phosphorous demands (Vielma and Lall, 1998; Vielma et al., 1999).

A single reference to histology by Day (1887; see discussion), repeated by Tchernavin, (1944, p. 228), no information about microstructure or histology of the kype is available. Since analysis of the structure and microstructure of bone and bone cells provides information about functional and metabolic properties of the skeleton (Smith-Vaniz et al., 1995), we studied the microstructural and underlying cellular processes involved in formation of the skeletal tissues of the kype. By providing basic data about the structure, growth, and dynamics of the kype, we seek both a better understanding of this unique skeletal structure and a basis for understanding its function. The present study profit, both from access to animals from one of the world's largest and best-studied populations of Atlantic salmon — that in the Miramichi River system in New Brunswick, Canada (Chaput, 1997; Chaput et al., 1998, 2000) — and from facilities which, for the first time, allowed processing of fresh tissue samples for various types of analysis.

**Results**

**Gross Morphology**

All 1SW male salmon (grilse or salmon that have spend one winter at sea before returning to spawn for the first time; see Table 1 for terminology) had a well-developed kype at the distal extremity of the lower jaw as an extension of the dentary bone (Fig. 1). Proximally, the kype is equipped with teeth but the teeth were partly covered by the oral epithelium when the fish were caught. Gross anatomical examination revealed that development of the kype involved morphological changes at the distal extremity of the lower jaw. The dentary is prolonged and curved dorsally. At the base of the kype, the height of the jaw increases (Fig. 2). No hard tissue is present at the tip of the kype, which is entirely composed of soft connective tissue (Fig. 2). When the mouth is closed, the kype fits into a deep cavity, located between the premaxillae. In grilse, the insertion of the kype into the upper jaw enables males to close their mouth, despite the presence of the kype (Fig. 2) Multi-sea winter salmon cannot close their mouths properly due to additional jaw alterations. Multi-sea winter salmon have been collected for a subsequent study.

**Radiology**

X-ray analysis of the dentary revealed that the hard tissue of the kype is composed distally of long skeletal needles that protrude distally and apically into the connective tissue of the kype (Fig. 3).

**Table 1**

<table>
<thead>
<tr>
<th>Terms</th>
<th>Definitions</th>
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<tbody>
<tr>
<td>Alevin</td>
<td>Yolk sac larvae</td>
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<tr>
<td>Fry</td>
<td>First feeding larva after the yolk sac has been resorbed</td>
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<tr>
<td>Parr</td>
<td>Juvenile salmon that stay for one to four years in freshwater</td>
</tr>
<tr>
<td>Mature Parr =</td>
<td></td>
</tr>
<tr>
<td>Smolts</td>
<td>Juvenile salmon, transferring from freshwater to saltwater</td>
</tr>
<tr>
<td>Landlocks</td>
<td>Males and females that do not migrate to the sea and become mature in freshwater</td>
</tr>
<tr>
<td>Grilse</td>
<td>Animals that return to their home rivers for spawning for the first time, after spending one winter in the sea (1SW = one sea winter salmon). Grilse usually have a fork length below 63 cm.</td>
</tr>
<tr>
<td>Salmon</td>
<td>Animals that return to their home rivers for spawning for the first time, after spending more than one winter at sea (MSW = multi sea winter salmon). Also animals that have survived the first spawning and that return to their home rivers for repeated spawning, after spending one or more “winters” (years) in the sea. Salmon entering fresh water also called “bright salmon”. The fork length of salmon is usually above 63 cm.</td>
</tr>
<tr>
<td>Kelts</td>
<td>Animals that have survived spawning. Kelts stay over winter in the river and return to the sea in the spring. Kelts are also called “black salmon”.</td>
</tr>
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In *Salmo salar*, animals from one population can enter different life cycles, dependent upon the age of maturation, duration of the fresh water and marine life phases, and the number of spawning events during the lifetime. To describe the different stages, terms are applied which are not commonly used in fish biology (Mills, 1989, Shear, 1992, Fleming, 1996). These terms, such as grilse and salmon, are defined above. A kype develops in male grilse and in male salmon. See Moore et al. (1995) for characteristics of the life cycle of animals from the Miramichie River system.
Shorter skeletal needles that extend in a ventral caudal direction broaden both the base of the kype and extend proximally into the distal part of the dentary. Skeletal needles are distinguishable from the compact bone of the dentary (Fig. 3). X-rays also show the presence of unerupted teeth associated with the new hard tissue.

Structures described so far occur in all males. Individuals differ only slightly concerning the detailed shape of the skeleton of the kype. Also, the arrangement of the breeding teeth on the hard tissue of the kype differs between individuals; the nature of the hard tissue is described below. Analysis of such variation will form part of a future study. The extent of skeletal alterations that develop in the jaws of males can be seen by comparing the male (Fig. 3) with the jaw of a female (Fig. 4) which only displays signs of rudimentary skeletal needles distally and ventrally but not apically.

**Microstructure**

A schematic overview of the tissues of the kype and their distribution is shown in Fig. 5. Sagittal sections throughout the tip of the lower jaw confirm the presence of the skeletal needles, seen on X-rays. The needles protrude distally into the connective tissue of the kype and anastomose proximally to form a spongiosa-like meshwork (Figs. 5, 6, 7). Thick skeletal needles, developing on the ventral side of the jaw, merge into a solid mass of hard tissue (Figs. 6, 8). As seen in X-rays, a border between the skeleton of the kype and the compact bone of the dentary is readily identifiable on the basis of organization of the bone structure (Figs. 5, 6).

**Skeletal Tissue and Cells**

The histological features of the skeleton of the kype and of the compact bone of the dentary are listed in Table 2; see also Fig. 5 for a schematic overview. At the tissue/cellular level the skeletal needles in both distal and ventral regions of the kype display features of rapidly growing bone. Growth is indicated by the presence of prominent osteoblasts (revealed by staining with Masson’s trichrome, Fig. 9), secretion of considerable osteoid (revealed by Masson’s trichrome and osteoid staining; Figs. 9, 10), delayed mineralization of osteoid (revealed by Masson’s trichrome and osteoid staining; Figs. 9, 10), and the presence
of proteoglycans in the zone of osteoid formation (revealed by HBQ, Toluidine Blue and AH staining; Fig. 11).

Rapid bone formation, however, does not lead to the development of typical periosteal bone. Distally, a modified form of intramembranous bone formation results in the development of a chondroid bone-like tissue as bone forming cells develop into osteocytes (surrounded by bone matrix and equipped with characteristic cell processes) and chondrocytes (Figs. 12, 13, and see Table 2 for the features used to identify cells as chondrocytes). Basically the skeletal needles contain a mixture of bone and cartilage cells but locally, bone cells or cartilage cells can predominate. Hyaline cartilage is present at the distal tip of the lower jaw, joining the left and right dentary bones (Fig. 14).

Toluidine blue-, Alcian blue/Haematoxylin-, and HBQ-staining methods provide evidence for the dual presence of osteocytes and chondrocytes within the bone matrix (Figs. 8,11,12,13). In addition,
Differentiation and Growth of the Salmon Kype

the presence of cartilage-specific extracellular matrix in the bone was verified by chondroitinase and hyaluronidase digestion, which specifically remove cartilaginous ground substances, in this case, in the vicinity of cells identified on histological and histochemical criteria as chondrocytes. Enzymatic digestion results in negative proteoglycan staining with Toluidine Blue and Alcian Blue/Haematoxylin (Fig. 15).

Chondrocytes inside the bone matrix could also be labeled with a monoclonal antibody directed against collagen type II, which is the typical collagen type of cartilage (Beresford, 1993). Bone cells did not react with the antibody (Fig. 17).

The periosteal hard tissue that develops ventrally in the kype is typical Sharpey fiber bone, i.e., a bony tissue with thick collag-
enous fiber bundles that extend from the connective tissue, in this case of the kype, into the new bone matrix. Large osteoblasts secrete osteoid into the gaps between the fibers (Figs. 17, 18). As in the deposition of skeletal needles distally, osteoblasts develop into typical osteocytes and chondrocytes (Fig. 19). Consequently, the ventral segment of the kype is Sharpey fiber bone that also displays features of chondroid bone similar to that described by Beresford (1993) as chondroid bone type one. HBQ- and Elastin-staining provide evidence that the fibers that continue from the connective tissue into the bone matrix are made of collagen. Elastin, recently reported to occur in fish bone (Miyake et al., 2001) is restricted to the connective tissue part of the kype and to the walls of blood vessels (Fig. 20).

**Mineralization**

As revealed by osteoid- and Masson’s trichrome-staining, the pattern of mineralization of the skeletal tissue of the kype varies according to type and location of the developing hard tissue.

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**Fig. 22.** Chondrocytes inside the skeleton of the kype mediate a cartilage-like mineralization (red staining) that starts in the vicinity of single cells and cell groups (black arrowhead). Pairs of chondrocytes indicate that cells continue to divide prior to matrix mineralization. Osteoid staining. Scale bar, 40 µm.

**Fig. 23.** The compact bone of the dentary (top) is clearly separated from the skeleton of the kype (below) which is composed of both Sharpey fiber and chondroid bone. The border between compact and Sharpey fiber bone is labeled by white arrowheads. An osteon (black arrowhead) is forming in the vicinity of the cortical bone. Masson’s trichrome staining. Scale bar, 150 µm.

**Fig. 24.** A primary osteon in the cortex of the dentary. Notice the absence of chondrocytes and Sharpey fibers in the bone matrix. The endosteal surface is covered with osteoid (blue staining, black arrowheads). Osteoid staining. Scale bar, 60 µm.

**Fig. 25.** A primary osteon in the cortex of the dentary. The circular arrangement of the parallel fiber bone (white arrowheads) has been visualized using polarized light microscopy. HBQ staining. Scale bar, 60 µm.

**Fig. 26.** Cross linkage of skeletal needles creates bone marrow space that primarily contains connective tissue (white arrowheads). Masson’s trichrome staining. Scale bar, 150 µm.

**Fig. 27.** Bone resorbing cells in salmon display typical features, also known from mammalian Osteoclasts. The cells express TRAP (red), are multinucleated and display a ruffled border (black arrowheads). Secreted TRAP appears as a red stripe at the bone surface (white arrowhead). The extracellular dephosphorylation of bone sialoprotein and osteopontin by TRAP is thought to be responsible for the observed detachment of the cells from the bone surface (Ek-Rylander et al., 1994) as is the case for the right osteoclast and for cells in Fig. 28. TRAP staining. Scale bar, 40 µm.

**Fig. 28.** An alternative way of creating bone marrow space is by bone resorption by multinucleated osteoclasts (black arrowheads). Along with the osteoclasts, blood vessels (white arrowheads) are present in the bone marrow. Osteoid staining. Scale bar, 60 µm.

**Fig. 29.** Remodeling of chondroid bone into compact bone, finally results in the formation of new osteons, made of circular arranged parallel fibered bone (white arrowheads). As in complete osteons (Fig. 24), the endosteal surface is covered by osteoid (black arrowhead). Osteoid staining. Scale bar, 60 µm.
Skeletal needles display a mineralization front that starts beneath the broad osteoid zone (Fig. 11). In the ventral Sharpey fiber bone, collagen fibers are the sites of first mineral deposition (Fig. 21). Cartilage and cartilaginous parts of the skeleton of the kype undergo a cartilage-like pattern of mineralization, starting in the vicinity of chondrocytes (Fig. 22).

**Bone Matrix**

The kype skeletal tissue is made from primary cancellous, chondroid-like bone distally (Figs. 11-15) and from Sharpey fiber bone with chondroid bone-like features ventrally (Figs. 17-22). These tissues clearly differ from the compact bone of the dentary, which consists of parallel-fibered bone, structurally organized in osteons (Figs. 23-25). Compact bone contains neither chondrocytes nor Sharpey fibers (Fig. 24). The border between the compact bone of the dentary and the skeletal tissue of the kype is characterized by an abrupt change of bone type. The chondroid-like bone and the chondroid-like Sharpey fiber bone in direct contact with the compact bone (Figs. 6,23). The abrupt structural changes allows cortical bone and skeletal needles of the kype to be discriminated in (Figs. 3,4).

**Remodeling**

The skeletal tissue of the kype displayed evidence of continuous growth at the time the salmon were caught. Growth includes development of cross connecting distal skeletal needles into a spongiosa-like formation (Figs. 6,7) which creates small bone marrow spaces containing connective tissue (Fig. 26). Concurrently, proximal parts of the skeleton of the kype are converted into compact bone (Fig. 21), which involves the creation of bone marrow space by osteoclastic bone resorption (Figs. 27-29), but not by cross connection of bone needles. From the analysis of serial sections we interpret this as a typical bone remodeling sequence involving the resorption of primary bone by numerous multinucleated and TRAP active osteoclasts (Figs. 27,28) and it's replacement by secondary lamellar bone (Fig. 29). Lamellar bone then forms the inner wall of forming osteons. Through bone remodeling, both the skeleton of the kype and the bone of the dentary increase and grow.

**Discussion**

The morphological alterations observed in the lower jaws of Atlantic salmon migrating upstream from the Miramichi river match the morphological descriptions of the phenomenon provided by Day (1987), Tchernavin (1938a) and Kacem et al. (1999) for Atlantic salmon migrating in Britain, Scotland and France. Hence, there is reason to assume that the alterations in the jaws of males found in this study are typical for Atlantic salmon in other locations, irrespective of population or genetic background.

Despite precise descriptions of the reinitiation of bone growth and morphological changes by Tchernavin (1938a) and Kacem et al. (1999), the hard tissue(s) of the kype have not been analyzed in detail. Darwin (1909, p. 510) discusses it as a cartilaginous projection, citing Yarrell's *History of British Fishes* as the source. Day (1887) cites Gadow as having sectioned a kype, stained it with carmine, and described it as consisting “entirely of fibrous connective tissue, without any traces of cartilaginous cells in it.” Day goes on: “Therefore the hook cannot be looked upon either as an outgrowth of the bones of the lower jaw, or as a sort of horny excrescence like horns, nails...” (p. 57). In the few subsequent studies that have commented on the tissue (none of which undertook any histological analysis), it has been regarded as bone (Tchernavin, 1938c, Persson et al., 1998). Our analysis reveals that the hard tissues of the kype are neither cartilage nor typical bone and are certainly not keratin as seen in horns or nails but differs from the compact bone of the dentary. The composition of the unique skeleton of the kype, its growth patterns, and its possible functional significance are discussed below.

**The Skeletal Tissues of the Kype**

The special characters of the skeletal tissues of the kype and their divergence from the compact bone of the dentary can be seen on high resolution X-rays, a technique that we and some others now regularly apply to detect skeletal alterations in teleosts (Witten et al., 1999b, Afonso et al., 2000). The border between new bone of the kype and of the dentary is clearly discernible on X-rays. Histological sections allow visualization of the microanatomy of the structures and confirm the radiological observations that proximally, compact bone of the dentary switches abruptly into a needle-like skeletal structure. The future application of X-radiography to analyze changes in the lower jaws of salmon in the field will allow analysis of large numbers of animals, and, with multiple X-rays through time, allow skeletal changes in individual animals to be followed. The use of such
a non-invasive technique on live animals is highly desirable in view of the world-wide declining stocks of wild Atlantic salmon. At the same time, there is an increasing need to gather information about the life history of the animals (Hutchings and Jones, 1998).

Apart from radiological and microstructural studies, histological and histochemical analyses confirm the specific character of the skeletal needles of the kype: (1) The needles display a rapid periosteal skeletal growth that differs from periosteal bone formation; (2) A loose meshwork of bony trabeculae then forms; (3) Subsequently, the tissue exhibits a character that is intermediate between bone and cartilage with Sharpey’s fibers ventrally and (4) Finally the skeleton of the kype is partly remodeled into compact bone without cartilage-like features and without Sharpey fibers.

Rapid skeletal growth in a life phase where the animals have stopped feeding and utilize as much as seventy percent of their energy reserves for metabolically expensive upstream migration, for gonad maturation, and for competition with other males on the spawning grounds (Fleming, 1996) is a remarkable event. It has been hypothesized that formation of the kype and the changes in bones of the skull that occur during migration requires resorption of minerals from the operculum, the vertebral column or the scales (Tchernavin, 1938b; Persson et al., 1998; Kacem et al., 2000). The growth pattern of the skeletal tissue of the kype suggests, however, that despite mineral support from other skeletal elements, the bony tissue is made from the least amount of material possible; the skeleton of the kype does not arise as a solid bone mass, but as a meshwork of anastomozing skeletal needles. The spongiosa-like structure of the resulting tissue likely provides a maximum of mechanical stability with a minimum of bone mass, as in spongiosa in the bone marrow of mammals (Parfitt, 1988).

The spongiosa-like periosteal bone tissue in the skull of other teleosts (hyperostotic bone) might serve a mechanical function or only play a role in mineral metabolism (Smith-Vaniz et al., 1995; Meunier and Desse, 1986). The rapid and structured growth of the skeleton of the kype as revealed by prominent osteoblasts, a broad osteoid zone, deposition of proteoglycans and expression of cartilage characteristics (Huysseune and Verraes, 1990; Beresford 1993; Nah et al., 2000), suggests that the skeleton of the kype provides a maximum of support in a physiological situation where somatic growth has stopped (Persson et al., 1998). Although, bone resorption occurs proximally in the kype, there is no indication that the skeleton of the kype functions as a mineral reserve in anadromous mammals, since this and other studies show that bone resorption is always linked to the concurrent formation of new bone (Witten et al., 2000).

The Function of the Kype and Kype Skeleton

So far, only a few studies have focused on the possible function of the kype. Tchenavin (1938c) regards the kype as “no good to the fish, and seems to be somewhat absurd, preventing as it does the use of the strong curved teeth which appear at the same time” (p. 37). Witten and Hall (2001) suggested that the kype is not merely a by-product of hormonal and environmental alterations. The kype might function as a secondary sexual character, either for competing with other males, or to attract females (Fleming, 1996; Hutchings and Myers, 1987; Järv, 1990). Darwin certainly thought so:

Male salmon have been observed fighting all day long [and that] special means of defense may be given through means of sexual selection, as the mane of the lion and the hooked jaw to the male salmon; for the shield may be as important for victory, as the sword or spear (Darwin, 1910, p. 64).

Ideas raised about functional and mechanical properties of the skeletal tissues of the kype are based on analysis of the hard tissue microstructure (Smith-Vaniz et al., 1995). Thus, the unique character of kype hard tissues should be discussed in more depth. The skeleton of the kype arises from the periosteum of the dentary but its structure is clearly divergent from compact bone of the lower jaw, which is made of parallel-fibered bone and osteons. In contrast, the skeleton of the kype consists of anastomozing skeletal needles made of woven bone and contains no osteons. The differences between the kype and the compact bone of the dentary are summarized in Table 2. Besides osteocytes, the bone matrix contains chondrocytes, while the ventral part of the skeleton of the kype also contains Sharpey fibers.

Antlers of deer are periosteal bone formations that share features with the skeleton of the kype. Antlers function as a secondary sexual character, arise from a specific site on a dermal skeletal element (the pedicel of the frontal bone), and both their structure and mode of formation differ from those of the frontal bone. There is an initial cartilaginous phase and metaphasic conversion of cartilage to bone in antlers. Rapid growth and the retention of cartilage characters are features shared with the skeleton of the kype (Hall, 1978; Goss, 1983; Szuwart et al., 1995, 1998).

In view of the astounding variety of skeletal tissue that occur in teleosts (Hall, 1978; Benjamín, 1988a,b; 1990, Huysseune, 2000) it is no surprise that secondary periosteal skeletal tissue is not only found in mammals and salmon but occurs in other fish species. Regular hyperostotic teleost bones—formerly regarded as pathological and referred to as an osteoma (Schunkberger and Lucke, 1948)—share features with the skeleton of the kype. Hyperostotic bones not only have a spongiosa-like appearance, but in teleosts with acellular bone they may represent areas of the skeleton that undergo remodeling (Smith-Vaniz et al., 1995). In contrast, salmon have cellular (osteocyte-containing) bone and the whole skeleton (endoskeleton plus dermal bones and scales) is thought to be metabolically active (Persson et al., 1998, 1999; Vielma and Lall, 1998; Kacem et al., 2000). However, like hyperostotic bones of advanced teleosts (Smith-Vaniz et al., 1995), in the upstream migrating salmon we examined, the skeletal tissue of the kype, not the compact bone of the dentary, displays remodeling, mediated by prominent multinucleated osteoclasts.

Bone and Cartilage Characters of the Kype Skeleton

The skeleton of the kype is formed by cells that resemble typical osteoblasts (Hall, 1978, 1990, 1991). In the ventral part, the cells secrete new bone matrix (osteoid) in gaps between the Sharpey fibers. Distally, membrane bone formation takes place. The two halves of the kype are united by hyaline cartilage, who’s location and lack of connection to Meckel’s cartilage would lead us to identify the tissue as secondary cartilage (Beresford, 1981). However, we do not yet know the developmental history of this cartilage. It could be a remnant of Meckel’s cartilage not connected to Meckel’s cartilage in adults; it would have to arise from periosteal cells to qualify as a secondary cartilage (see Benjamín, 1988b).

Interestingly, not all cells that derive from osteoblasts and become entrapped in the bone matrix develop into osteocytes. Cells that have all the features of osteoblasts also develop into typical chondrocytes, the latter identified as being rounded, show-
ging cell division, being arranged in rows and territories, producing cartilage matrix and displaying a cartilage-like pattern of matrix mineralization (Moss and Moss-Salentijn, 1983; Huysseune, 2000). Given that the skeleton of the kype entirely derives from intramembranous ossification, the appearance of cartilage characters is remarkable. However, it is now established that the expression of cartilaginous features is a general character of growing membrane bones (Fang and Hall, 1997, 1999; Nah et al., 2000). Furthermore, there is strong evidence that osteoblasts and chondroblasts are members of the same class of scleroblasts (Hall, 1970, 1978, 1991; Hall and Miyake, 2000). The mechanisms discussed above are likely also involved in the development of chondroid bone, a tissue that occurs in several teleost species and that shares morphological and histochemical features with the skeleton of the kype (Beresford, 1981; Benjamin, 1990; Huysseune and Verraes, 1990; Taylor et al., 1994). Similar to chondroid bone in cichlids, the growth of the kype bone involves osteoblasts that acquire characteristics of chondrocytes as they become trapped in the bone matrix. The idea has been raised that chondroid bone is an adaptation to rapid bone growth (Hall, 1990; Huysseune, 1990; Taylor et al., 1994), an interpretation that applies to both the distal and the ventral parts of the skeleton of the kype.

**Sharpey Fiber Bone in the Kype Skeleton**

Apart from displaying chondroid bone-like characters, the ventral part of the skeleton of the kype is also a Sharpey fiber bone. A similar tissue, that displays features of chondroid bone and of Sharpey fiber bone, has not been described in teleosts previously. Sharpey fiber bone - characterized by the continuation of collagen fiber bundles from connective tissue into the bone matrix - is commonly associated with joints and teeth, providing a strong link between connective tissue and the skeleton (Beresford, 1981; McKee et al., 1996). Hence, the presence of Sharpey fiber bone in the ventral part of the skeleton of the kype further supports the idea that this skeletal tissue is functionally integrated in the architecture of the salmon jaw. It is not a by-product of physiological changes but serves a mechanical function.

Appearance of Sharpey fiber bone in the dentary is not restricted to the kype of grilse. Hughes et al. (1994a, b) described Sharpey fiber bone in the lower jaws of juvenile salmon. Interestingly, their description of Sharpey fiber bone in the lower jaw of perciform teleosts (Sparidae) also matches the tissue found in salmon. In distinction from Hughes et al. (1994b), who identified all cells inside the Sharpey fiber bone as osteocytes, we conclude that the Sharpey fiber bone of salmon contains chondrocytes. If the cells inside the Sharpey fiber bone of Sparidae that display a cartilage-like morphology are chondrocytes and not osteocytes (as we suspect from the figures published by Hughes et al., 1994b), then the type of hard tissue in the kype ventrally is not restricted to salmon.

**The Kype and Salmon Life History**

The consequences of different life strategies of male Atlantic salmon that range from premature kype-less paar to kype-bearing mature male salmon are not fully understood (Shear, 1992; Moore and Waring, 1999; Jones and Hutchings, 2001). Mature males invest heavily in the spawning success, and the development of a prominent kype is a part of the male investment, resulting in an increased mortality (Fleming, 1996). As suggested by Tchernavin (1938c), the kype may become a disadvantage for anadromous males (imparing proper feeding, breathing and swimming) after fulfilling its purpose as a secondary sexual character. Although, the microstructure of the kype tissue is different from the bone of the jaws, developmental processes and hard tissue elements of the kype are known from the dermal skeleton of mammals and other teleost species. Finally, the rapid growth of the skeleton of the kype appears to be a regulated process, integrated in the development of the dentary, and understandable in the light of the general strategy of Atlantic salmon to survive spawning. The growth of compact bone, through remodeling of the skeleton of the kype seems to produce non-reversible alterations of the dentary in repetitively spawning individuals. However, it remains to be clarified if the changes gradually disappear after spawning and if the dentary resumes its former shape and proportions in those males which return to sea, as hypothesized by Tchernavin (1937). Future studies will address the extent of the process and its limits for the fitness of individual anadromous male salmon.

**Materials and Methods**

**Sampling**

Ten male anadromous grilse (see Table 1 for definition of terms), collected during the fall run in 1999 and 2000 (September) at the southwest Miramichi River above Red Bank (46°56.8’ N, 65°48.3’ W, New Brunswick, Canada) were used for this study. Animals were caught in traps used for the Red Bank (First Nation) food fishery. The animals were caught and sampled under license from the Canadian Federal Department of Fisheries and Oceans.

Weight and fork length were determined in order to calculate the condition factor \( K = \frac{\text{weight (g)}}{\text{fork length (cm)}^3} \times 100 \) of the fish (Andrt et al., 1996). Scales were taken to evaluate the animals life history; part of a Department of Fisheries and Oceans monitoring program. All grilse used for this study were in “good shape,” as determined by gross morphological inspection, and showed no signs of diseases. As revealed by scale reading (Shearer, 1992), all animals had spend one winter in the ocean (one sea winter salmon = 1SW; see Table 1) before returning to their home river. The fork length of males varied between 55.4 and 63.5 cm, weight ranged from 2010g to 2500g. Condition factors were calculated as between 0.93 and 1.17 which is in the range of the mean condition factor \( K = 1.02 \) for males from this age group in the Miramichi river system (Moore et al., 1995).

For gross anatomical documentation external picture of the entire head as well as pictures of half heads were taken. Subsequently, the jaws were x-rayed, processed and immediately fixed for seven different analytical procedures.

**X-ray Analysis**

High resolution X-rays of the left halves of all fish heads were taken using a portable “Mini X-ray HF80+” machine (Mini X-ray inc., Northbrook, USA) and “Kodak Industrex M Film Ready Pack II” (Kodak Industry, France) without a screen. The settings of the X-ray unit were 70 kV, 15 mA, 2 sec exposure time, and a distance of 40 cm between the beam source and the X-ray film. Radiographs were developed with Kodak chemicals and analyzed using a stereo microscope.

**Basic Histological Procedures**

Immediately after capture, right jaws were fixed in 10% neutral buffered paraformaldehyde for 24 hrs, rinsed in tap water for 24 hrs, and decalcified for 72 hours in a 10% EDTA solution buffered with 0.1 M TRIS base, pH 7.0. After decalcification, samples were dehydrated in a graded series of ethanol and subsequently embedded in Paraplast. Starting at the midline 10 µm sagittal sections were prepared and stained with Masson’s Trichrome (Flint and Lyons, 1975) for demonstration of collagen, bone cells, and mineralized bone and with the Hall-Brunt
Quadraple Stain (HBQ) (Hall, 1986) to differentiate bone, cartilage, chondroid bone and to identify collagen fibers. Sections were mounted with DPX (Fluka).

**Additional Histological and Histochemical Analyses**

Additional analytical procedures were applied to demonstrate osteoid and the pattern of mineralization, visualize bone remodeling, identify chondrocytes and cartilage matrix within bone, and to discriminate among elastin and collagen fibers.

To differentiate osteoid (non-mineralized bone matrix) and mineralized bone matrix and to reveal the pattern of mineralization, osteoid staining was performed, following the trichrome method of Rails and Watkins (1992).

To demonstrate bone remodeling, decalcified, sections were stained with Toluidine Blue as follows: Descending ethanol series (100% ethanol to water); 1% aqueous Toluidine Blue solution (pH 4.5) for 45 minutes; differentiation and dehydration in 100% Ethanol; mounting.

To identify osteoclasts and osteoclasts activity, tartrate resistant acid phosphatase was demonstrated (TRAP) following the protocol of Witten et al., 2001. Briefly, samples were (a) fixed in 10% formaldehyde (buffered to pH 7.2); (b) rinsed in tap water for 1 hr; (c) decalcified with EDTA (see above), (d) dehydrated in graded acetone solutions and (e) embedded in glycol-methacrylate. Sections of 5 µm were mounted on uncoated slides. For demonstration of TRAP activity, specimens were preincubated for 30 min at 20°C in 100 mmol acetate buffer + 50 mmol L⁻¹ di-sodium tartrate dihydrate at pH 4.5. Staining was performed in the presence of tartrate using naphthol AS-TR phosphate as substrate (final concentration 170 mmol/L) and hexazotised paraarsoniliane (final concentration 1,58 mmol/L) as an azo-coupling dye. Specimens were counterstained with Mayer’s haematoxylin. Control procedures revealed the specificity of the staining and consisted of: (a) heating at 90°C for 10 min prior to staining, (b) incubation without substrate, (c) adding the inhibitor NaF to the staining solution (10 mmol l⁻¹).

For the selective demonstration of cartilage matrix proteoglycans, sections were stained with a combined Alcian Blue/Haematoxylin staining (AH). Deparaffinized sections were treated with 3% acetic acid for 3 min, sections at 4°C for 12 hrs. Slides were flushed with PBS buffer and the staining and consisted of: (a) incubation with a non-specific mouse IgG antibody, (b) incubation only with the alkaline phosphatase-labelled secondary antibody, and (c) incubation with ALP substrate only.

Since elastin was recently described as a component of bone and teeth in fish (Miyake et al., 1999, 2001) the possible presence of elastin fibers in the hard tissues of the kype was evaluated using the Verhoeff staining procedure (Presnell and Schreibman, 1997).

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